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The effect of fatty acids and starvation on the metabolism of gluconeogenic precursors by isolated sheep liver cells

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Isolated liver cells prepared from fed sheep synthesize glucose from propionate at twice the rate observed with cells from starved animals. Addition of palmitate or palmitate+carnitine to incubations of liver cells from starved animals inhibited the rate of glucose synthesis with lactate as a precursor, but had little effect when propionate and pyruvate were substrates. Liver cells from fed and starved sheep synthesized lactate and pyruvate when incubated with propionate. Fatty acids inhibited this formation of lactate and pyruvate from propionate. It is proposed that the different responses of gluconeogenic precursors to fatty acids can be explained by the effect of reducing equivalents on the transport of carbon atoms across the mitochondrial membrane.

INTRODUCTION

Studies on the regulation of hepatic gluconeogenesis have revealed major species differences in the relative rates of gluconeogenesis from various precursors (Elliott *et al.*, 1976). Propionate is utilized by hepatocytes from guinea pigs and sheep to a much greater extent than by cells from rats and mice. Lactate is, conversely, a poorer substrate in the former two species, and this presumably reflects the herbivorous diet and the fermentation of ingested carbohydrate to volatile fatty acids, including propionate (Pogson *et al.*, 1983).

The distribution of the activity of phosphoenolpyruvate carboxykinase [GTP:oxaloacetate carboxylyase (transphosphorylating), EC. 4.1.1.32] between the cytosolic and mitochondrial compartments varies from species to species (Hanson, 1980; Hanson & Garber, 1972). In rat hepatocytes utilizing pyruvate as a substrate, malate efflux from the mitochondria satisfies both the carbon and reducing-equivalent requirements for gluconeogenesis, because phosphoenolpyruvate carboxykinase is localized in the cytosol (Nordlie & Lardy, 1963). In contrast, in guinea-pig and rabbit livers, where phosphoenolpyruvate carboxykinase activity is found in both mitochondrial and cytosolic compartments (Nordlie & Lardy, 1963), gluconeogenic carbon atoms can arise as a result of mitochondrial phosphoenolpyruvate generation, and the availability of reducing equivalents may be an important factor in gluconeogenesis from propionate or pyruvate (Zaleski & Bryła, 1979). Fatty acids stimulate gluconeogenesis from lactate or pyruvate in rats (Williamson *et al.*, 1969), whereas, in the guinea pig or rabbit, fatty acids may either inhibit or stimulate glucose synthesis, depending on the substrate and sensitivity to intramitochondrial redox changes (Zaleski & Bryła, 1977; Söling *et al.*, 1973).

In ruminant liver, hepatic gluconeogenesis is a major metabolic activity in the fed animal, and decreases during starvation (Bergman *et al.*, 1970; Lomax & Baird, 1983). This is in contrast with most species, which respond to starvation by increasing gluconeogenic flux. We have carried out a study which examines the regulation of gluconeogenesis from different substrates in response to starvation or fatty acids in sheep liver. The results indicate that the gluconeogenic pathway in sheep liver is influenced by fatty acids in a similar manner to that in other herbivorous species, but that the metabolism of propionate to lactate may have a particular significance in ruminants.

EXPERIMENTAL

Animals

Sheep were 1-year-old castrated males and were either pasture-fed (during the summer period) or fed on a hay diet with barley concentrate (during winter).

Chemicals and enzymes

Enzymes and biochemicals were purchased from Boehringer Corp. (London), Lewes, Sussex, U.K., with the following exceptions. Glucose oxidase (type II), sodium palmitate and sodium octanoate were from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K., and peroxidase was from International Enzymes, Windsor, Berks., U.K. L-Carnitine was a gift from Dr. G. D. Baird, Institute for Research into Animal Diseases, Compton, Berks., U.K. Bovine serum albumin (fraction V; Miles Laboratories, Slough, Berks., U.K.) was freed of fatty acids and other ligands by the method of Chen (1967).

All other chemicals were of the purest grade available from standard suppliers.

Incubation conditions and assays

Isolated liver cells were prepared from sheep by perfusion of the caudate lobe with buffer containing collagenase as described by Lomax *et al.* (1983). Liver cells were dispersed and washed in buffer containing Ca^{2+} and 2% (w/v) albumin. Metabolic integrity of cell

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Table 1. Effects of fatty acids and of starvation on the synthesis of glucose from various precursors and the [3-hydroxybutyrate]/[acetoacetate] ratio in sheep liver cells

Incubations, assays and calculations were as described in the text. Results for fed sheep are corrected for changes in cellular glycogen during incubation. Sheep were starved for 4 days. Results are means ± S.E.M., for the numbers of independent observations given in parentheses. Significance between means (presence of fatty acids versus control) were assessed by paired *t* test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Substrate (all final concn. 10 mM)	Additions (final concn. 1 mM)	Glucose synthesis (nmol/h per mg dry wt.)		[Hydroxybutyrate] / [acetoacetate]
		Fed	Starved	Starved
None	None	155.7 ± 58.0 (5)	7.6 ± 2.9 (11)	0.26 ± 0.05 (9)
Propionate	None	338.2 ± 44.0 (5)	124.6 ± 9.5 (10)	0.38 ± 0.08 (7)
	Palmitate		134.2 ± 10.4 (6)	1.56 ± 0.23*** (4)
	Palmitate plus carnitine		133.3 ± 13.2 (6)	1.20 ± 0.30** (3)
	Octanoate		190.3 ± 37.3* (5)	1.86 ± 0.32*** (3)
Pyruvate	None		77.5 ± 8.1 (5)	0.29 ± 0.32 (2)
	Palmitate		113.2 ± 15.8* (4)	0.87 ± 0.06 (4)
	Palmitate plus carnitine		87.8 ± 9.7 (4)	1.05 ± 0.14 (3)
	Octanoate		136.7 ± 17.3* (5)	1.27 ± 0.21 (4)
Lactate	None		74.7 ± 13.7 (5)	
	Palmitate		35.0 ± 5.2 (3)	
	Palmitate plus carnitine		29.0 ± 4.8* (4)	
	Octanoate		25.6 ± 8.0* (4)	
Fructose	None		246.2 ± 19.5 (5)	
	Palmitate		239.9 ± 30.0 (3)	
	Palmitate plus carnitine		217.9 ± 30.2 (4)	
	Octanoate		255.8 ± 44.3 (4)	
Glycerol	None		15.1 ± 4.9 (6)	
	Palmitate		21.5 ± 3.8 (3)	
	Palmitate plus carnitine		17.9 ± 4.1 (4)	
	Octanoate		22.4 ± 4.7 (4)	
Alanine	None		10.7 ± 10.6 (3)	

preparations was assessed by measurement of cellular ATP content (Donaldson *et al.*, 1979).

Cells (5–6 mg dry wt./vial) in a final volume of 2 ml of buffer containing 2% (w/v) albumin were incubated as described by Lomax *et al.* (1983). In experiments with palmitate, the substrate was bound to albumin (Garland & Randle, 1964) and was present throughout the incubation period.

Glucose was determined enzymically (Krebs *et al.*, 1963), as were lactate (Gutmann & Wahlefeld, 1974) and pyruvate (Czok & Lamprecht, 1974). The concentrations of ketone bodies were determined as previously described (Mellanby & Williamson, 1974; Williamson & Mellanby, 1974).

Metabolic rates were not linear under all conditions; therefore metabolic activity is expressed as the amount of product accumulated between 0 and 60 min.

RESULTS AND DISCUSSION

The results in Table 1 demonstrate the importance of propionate as a gluconeogenic precursor in sheep liver. In starved sheep the capacity of the liver to synthesize glucose from propionate was significantly decreased ($P < 0.001$). Hepatocytes from starved sheep synthesized glucose from pyruvate and lactate at 40% of the rate from propionate. However, the net rate of glucose synthesis from lactate was higher than has been reported in hepatocytes prepared from sheep starved for 24 h (Demigné *et al.*, 1986*a*). These effects of starvation are consistent with studies *in vivo*, which have indicated that lactate replaces propionate in importance as a gluconeogenic precursor when propionate supply from the rumen is decreased during starvation (Baird *et al.*, 1980).

Hepatocytes from starved sheep synthesized glucose from fructose at twice the rate from propionate (Table 1). The rates of glucose synthesis from glycerol and alanine were very low, in agreement with the results of Ash & Pogson (1977).

Table 1 also shows the effects of adding fatty acids on the rate of glucose release from various precursors in hepatocytes from starved sheep. Palmitate or palmitate plus carnitine appeared to have little effect on glucose synthesis in the presence of either propionate or pyruvate. This result is similar to a report by Demigné *et al.* (1986*b*) that oleate does not influence either propionate uptake or synthesis to glucose by hepatocytes from 24 h-starved sheep. However, Table 1 shows that octanoate stimulated gluconeogenesis with either pyruvate or propionate as substrate, whereas all fatty acids inhibited glucose synthesis from lactate.

This pattern of stimulatory and inhibitory actions of fatty acids on gluconeogenesis has been observed in species that possess phosphoenolpyruvate carboxykinase activity in both cytoplasmic and mitochondrial compartments (Arinze & Hanson, 1973; Jomain-Baum & Hanson, 1975; Zaleski & Bryła, 1977). In these species it is thought that fatty acids induce changes in cellular

Table 2. Effects of fatty acids on the rates of lactate and pyruvate synthesis and on the [lactate]/[pyruvate] ratio in isolated liver cells from fed and starved sheep

Incubations, assays and calculations were as described in the text. Sheep were starved for 4 days. Results are means ± S.E.M., for the numbers of independent observations given in parentheses. Significances between means (presence of fatty acids or metabolic modulators versus control) were assessed by *t* test: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Substrate (final concn. 10 mM)	Additions (final concn. 1 mM unless otherwise stated)	Lactate synthesis (nmol/h per mg dry wt.)		Pyruvate synthesis (nmol/h per mg dry wt.)	[Lactate]/[pyruvate]
		Fed	Starved	Fed	Fed
None	None	−5.3±2.5 (5)	−2.2±3.0 (3)	1.9±1.3 (3)	10.3±5.0 (3)
Propionate	None	81.2±9.9 (5)	65.0±13.1 (3)	20.7±1.3 (3)	5.8±0.4 (3)
	Palmitate	67.8±11.4 (6)	24.1±9.0 (3)	8.2±2.3** (3)	7.9±0.8 (3)
	Palmitate plus carnitine	23.4±8.0*** (6)	8.0±7.4 (3)	3.8±0.8*** (3)	9.8±1.1* (3)
	Octanoate	36.3±12.2* (3)	—	6.6±2.3** (3)	9.5±1.0* (3)
	Butyrate (10 mM)	8.0±3.4** (3)	—	3.4±1.4*** (3)	8.7±2.1 (3)

redox state, which influence gluconeogenesis by altering the transport of carbon atoms across the mitochondrial membrane. In sheep liver, propionate carbon leaves the mitochondria as malate (Smith & Osborne-White, 1971), and phosphoenolpyruvate carboxykinase is present in both cytoplasmic and mitochondrial compartments (Ash & Pogson, 1977). The results of the present work suggest that fatty acids cause a rise in the mitochondrial [NADH]/[NAD^+] ratio, as indicated by the increase in the [3-hydroxybutyrate]/[acetoacetate] ratio (Table 1). This redox change would be expected to increase the rate of mitochondrial malate efflux and therefore stimulate the entry of pyruvate and propionate carbon into the gluconeogenic pathway. However, with lactate as the substrate, an increase in mitochondrial [NADH]/[NAD^+] ratio would be expected to increase oxaloacetate conversion into malate and result in a decrease in the flux of lactate carbon leaving the mitochondria as phosphoenolpyruvate or aspartate for gluconeogenesis (Jomain-Baum & Hanson, 1975). It therefore appears that sheep are similar to other species in which phosphoenolpyruvate carboxykinase is found in both cytoplasm and mitochondria, with respect to the influence of reducing equivalents produced by fatty acid oxidation on gluconeogenesis.

Hepatocytes prepared from both fed and starved sheep formed pyruvate and lactate when incubated in the presence of propionate (Table 2). There was no effect of starvation on the rate of lactate formation, and in fed sheep hepatocytes formed lactate approx. 4 times faster than pyruvate (Table 2). Significant rates of lactate and pyruvate synthesis in incubations with propionate have also been reported in sheep liver slices (Leng & Annison, 1963), in perfused liver (Richardson *et al.*, 1982) and in rumen epithelium (Pennington & Sutherland, 1956; Weekes, 1972; Leighton, 1984). This pathway of propionate metabolism may be associated with ruminants, because there is little evidence of conversion of propionate carbon into lactate and pyruvate in rat liver (Chan & Freedland, 1972).

Comparison of the results in Tables 1 and 2 shows that long- and medium-chain fatty acids inhibited propionate conversion into lactate plus pyruvate, but not conversion into glucose. These different effects on glucose and lactate formation are somewhat surprising, since the metabolism of propionate would be expected to share a common pathway up to the fate of cytoplasmic phosphoenolpyruvate; they are, however, consistent with an inhibition of pyruvate kinase (EC 2.7.1.40) when hepatocytes are incubated in the presence of fatty acids.

Alternatively, the pathway from propionate to lactate may involve malate efflux from the mitochondria together with either cytosolic NAD-dependent malic enzyme (EC 1.1.1.39) or malate dehydrogenase (EC 1.1.1.37) plus oxaloacetate decarboxylase (EC 4.1.1.3). Young *et al.* (1969) have proposed that an NADP-linked malic enzyme (EC 1.1.1.40) is responsible for lactate formation from propionate in rumen epithelium. However, this enzyme would not generate the cytosolic reducing power for lactate formation and, in any case, is virtually absent from sheep liver (Smith & Osborne-White, 1971).

The oxidation of propionate carbon to CO_2 must first involve metabolism to pyruvate, since precursors of tricarboxylic-acid-cycle intermediates cannot contribute carbon to CO_2 on a net basis. The high rate of pyruvate and lactate formation from propionate in the present study may well be related to the necessity to oxidize propionate to meet the energy requirements of the hepatocytes. The addition of fatty acids to hepatocyte incubations may then provide an alternative energy source and result in a decrease in propionate metabolism to lactate and pyruvate.

In sheep livers perfused with propionate or propionate plus alanine, significant amounts of the short-chain fatty acid appear to be oxidized, since these substrates stimulate oxygen uptake (Richardson *et al.*, 1982; Linzell *et al.*, 1971). However, studies *in vivo* have indicated that most of the propionate carbon must be used in glucose synthesis in order to balance the hepatic uptake of gluconeogenic precursors with output of glucose (Lindsay, 1978; Lomax & Baird, 1983). This evidence suggests that the control of the distribution of propionate carbon between oxidation and gluconeogenic pathways normally favours gluconeogenesis and could only assume physiological significance if the animals are fed on diets which give rise to very high propionate concentrations in the portal vein (Elliot, 1980).

Note added in proof (received 22 September 1986)

Faulkner & Pollock (1986) have reported the inhibitory effects of fatty acids on production of lactate and pyruvate by sheep hepatocytes incubated with propionate.

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